

The impact of two-component sensorial network in staphylococcal speciation

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Bacteria use two-component systems (TCSs) to sense and respond to their environments. Free-living bacteria usually contain dozens of TCSs, each of them responsible for sensing and responding to a different range of signals. Differences in the content of two-component systems are related with the capacity of the bacteria to colonize different niches or improve the efficiency to grow under the conditions of the existing niche. This review highlights differences in the TCS content between *Staphylococcus aureus* and *Staphylococcus saprophyticus* as a case study to exemplify how the ability to sense and respond to the environment is relevant for bacterial capacity to colonize and survive in/on different body surfaces.

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Current Opinion in Microbiology 2020, 55:xx–yy

This review comes from a themed issue on **Cell regulation**

Edited by **Charles J Dorman** and **Joan Geoghehan**

<https://doi.org/10.1016/j.mib.2020.02.004>

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Introduction

Bacterial genomes encode for a variable number of two-component sensor – response regulator pairs. The number of TCSs is proportional to the genome size, the diversity of environments in which organisms live, and the complexity in cellular differentiation [1]. Thus, bacteria with larger genomes, more metabolic versatility, and complex lifestyles are more likely to have a larger number of two-component systems than bacteria inhabiting relatively stable environments [2]. Acquisition of new TCSs occurs through mechanisms of lateral gene transfer or gene duplication and subsequent accumulation of mutations that insulate the new pathways from the existing two-component pathways [3]. In few cases, the newly introduced genes will improve the

efficiency to grow under the conditions of the existing niche and consequently will be fixed in the genome. In most of the cases, their presence will interfere with existing TCSs and they will be eliminated from the genome and thus, no longer present in extant species. Comparative analysis of the collection of TCSs present in two closely related bacterial species can be useful to explain why one bacterial species can colonize a wide range of tissues and cause many different types of infections, while the other is far more restricted in its distribution and pathogenicity [1,4].

Staphylococcus aureus is a highly versatile opportunistic pathogen able to adapt to very different types of environments. It can live freely outside the host or exist either as a commensal external colonizer or as a pathogen in both humans and animals [5]. The anterior nares are the main ecological niche for *S. aureus* [6]. However, multiple other sites in the human body such as the skin, axillae, vagina, and gastrointestinal tract can also be colonized by this bacterium. The core genome of *S. aureus* contains 16 TCSs (<http://mistdb.com>, http://www.ncbi.nlm.nih.gov/Complete_Genomes/SignalCensus.html, <http://www.p2cs.org>) [7,8,9*]. Among the sixteen TCSs, only WalRK, is essential for bacterial growth [10]. All the other TCSs are dispensable, and they can be deleted individually [11] or simultaneously in the same strain without affecting cell viability [12**]. Using *S. aureus* mutant strains deprived of its complete non-essential sensorial TCS network, Villanueva *et al.* [12**] showed that complementation with unique TCSs was sufficient to restore the capacity to grow under different environmental conditions such as low pH (GraRS) and low temperature (SrrBA) and to reduce nitrate to nitrite (NreCB) or to resist to Triton X-100 (VraRS), experimentally validating the widely offered idea that TCSs are self-sufficient, autonomous entities able to confer the capacity to sense and respond to a particular environmental condition. This study also showed that sensor histidine kinases exhibit strong preference for their cognate response regulators (RR), though in some cases, cross-regulation between non-cognate sensor-RR pairs can occur *in vivo*.

The set of 16 TCSs of *S. aureus* are conserved in other closely related coagulase negative staphylococcal species such as *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* (<https://mistdb.co>) [9*]. However, *Staphylococcus saprophyticus*, a coagulase negative staphylococcus whose genome is just 0.3 Mb smaller than that of *S. aureus*,

contains only 11 TCSs (Table 1) [13]. This lack of correlation between the genome size and the number of TCSs is very likely due to various environmental factors. *S. saprophyticus* is a common inhabitant of the urinary tract, perineum, rectum, urethra, cervix, and gastrointestinal tract [14]. It is the second most common cause of community-acquired urinary tract infections (UTI) in young and middle-aged female outpatients, after *Escherichia coli*, without the involvement of indwelling catheters [15,16]. The narrow niche of tissues that *S. saprophyticus* colonizes compared to *S. aureus* is very likely related with the reduced number of TCSs in *S. saprophyticus* and consequently to the capacity of the bacterium to adapt to the environmental conditions encountered in the different tissues [17]. In this review, we summarize and discuss our current knowledge about the TCSs that are missing in the *S. saprophyticus* genome compared to *S. aureus* and the consequences that their lack has for the bacterium.

KdpDE

In general *S. aureus* can tolerate a high concentration of salt and low water activity for a non-halophilic bacterium [18]. It is assumed that this osmotolerance supports bacterial growth on a high-salt environment such as the human skin. Potassium is the major monovalent cation in cells and plays an essential role for all living organisms. Within bacterial cells, potassium is required for the maintenance of a constant pH, membrane potential and osmotic pressure. *S. aureus* maintains high intracellular potassium concentrations of 0.5–1.5 M, even in the absence of a high osmolarity environment, thanks to two specific potassium uptake systems, the inducible Kdp and the constitutively expressed Ktr [19]. The activation of Kdp requires the presence of the functional KdpDE TCS which is induced by high osmolarity and inhibited by cyclic di-AMP [20]. Once activated, the most highly induced genes by the KdpDE TCS are the constituents of the KdpFABC transport machinery involved in uptake of K⁺, as well as genes involved in other compatible solute and sugar uptake; capsule biosynthesis; and amino acid and central metabolism (Figure 1). The KdpFABC system plays a physiological role under very low K⁺ conditions [21]. At high K⁺ concentrations, a lower-affinity and constitutively expressed Ktr ion transporter is responsible for K⁺ transport using energy generated by electrochemical ion gradients [22]. The analysis of mutants in Kdp and Ktr systems revealed that the Ktr system is the major K⁺ uptake system in *S. aureus* and the function of Kdp is required primarily during times of K⁺ starvation and/or fluctuating ionic conditions. However, acquisition of potassium ions in a potassium-limited environment seems to be important during *S. aureus* infection [23]. Accordingly, some methicillin resistant *S. aureus* strains carry a second KdpDE homologous TCS in the SCCmec mobile element [24]. Similarly, a KdpDE paralog has also been described in some types of the arginine catabolic mobile element (ACME) of *S. epidermidis* [25,26]. In some *S. epidermidis* strains containing the KdpDE system of the ACME element,

the chromosomal KdpDE copy has been lost very likely to avoid cross-talk between both systems. *S. saprophyticus* has to cope with the highly variable ion content of urine without the contribution of the KdpDE system. In this bacterium, osmotolerance relies on Ktr and other osmoprotectant transport systems (proline/betaine, glycine betaine/choline transporter, proline permease) (Figure 2) [13]. Because urine contains large amounts of potassium, it seems that the Ktr system is sufficient to import enough K⁺ in the absence of the high affinity uptake system. Another interesting peculiarity regarding the cellular osmotic tolerance of *S. saprophyticus* and not *S. aureus* is the presence of plasmids carrying the aquaporin gene (*aqpZ*). Aquaporins are water channels that mediate rapid entry or exit of water in response to changes in osmolarity, and consequently, it has been proposed that they may also aid to osmotic balancing [13]. However, this hypothesis remains to be tested.

SaeSR

The staphylococcal accessory element (*sae*) TCS has been previously characterized as a positive regulator of many secreted toxins, exoenzymes, and immunomodulatory proteins involved in staphylococcal pathogenesis (Figure 1). Loss of SaeSR abolishes the secretion of many of these proteins including exotoxins, immune evasion factors, superantigens, adhesins, staphylocoagulase, and proteases [27]. All these factors have a direct causative link with *S. aureus* pathogenicity and play a role once bacteria have crossed the epithelial barrier by inhibiting complement activation, neutrophil recruitment, as well as blocking opsonization by immunoglobulins [28,29]. It is therefore not surprising that mutants lacking *saeSR* result in lower mortality in systemic and intraperitoneal mouse infection models and show reduced ability to adhere to lung epithelial cells [30,31]. *S. saprophyticus* infections affect almost exclusively the urinary tract, without crossing the epithelial barrier and consequently, most of the typical virulence factors regulated by SaeSR in *S. aureus* are not needed in *S. saprophyticus* lifestyle. Instead, *S. saprophyticus* needs to maintain tight adherence to the bladder and ureter epithelium. For that, it produces different types of adhesins such as the highly conserved Aas hemagglutinin, the surface-associated lipase, Ssp, that forms fimbria-like surface appendages, and cell wall-anchored proteins (UafA, UafB, SssF and SdrI) [13,25,26] (Figure 2). UafA is a chromosome-encoded adhesin that mediates hemagglutination and adherence to human bladder cells. This adhesin is exclusive of *S. saprophyticus* strains and it is absent in other staphylococci. UafB is a plasmid encoded serine-rich glycoprotein that binds fibronectin, fibrinogen, and human bladder-epithelial cells. SssF is another plasmid-encoded cell-wall associated protein that is highly prevalent in clinical isolates. It is associated with resistance to linoleic acid, a component of the human skin innate immune defense system. It has been proposed that SssF may aid to the survival on the skin during non-UTI periods [32]. SdrI is a plasmid-encoded cell wall-associated serine-aspartate-rich protein that binds collagen and plays a role in acute UTI and

Table 1

Two-component systems in *S. aureus* and *S. saprophyticus*

Two-component system	<i>S. aureus</i>	<i>S. saprophyticus</i>	Function
<i>walRK</i>	MW0018 MW0019	SSP0021 SSP0022	Cell wall maintenance, cell viability
<i>hptSR</i>	MW0198 MW0199		Intracellular survival, uptake of hexose phosphate
<i>lytSR</i>	MW0236 MW0237	SSP0463 SSP0464	Autolysis, eDNA release, biofilm
<i>graRS</i>	MW0621 MW0622	SSP2061 SSP2062	AMPs resistance, growth at low pH
<i>saeSR</i>	MW0667 MW0668		Virulence factors regulation (toxins, exoenzymes . . .)
<i>tcs7SR</i>	MW1208 MW1209	SSP1446 SSP1547	Uncharacterized function
<i>arlSR</i>	MW1304 MW1305	SSP1323 SSP1324	Pathogenesis mechanisms: autolysis, adhesion, biofilm . . .
<i>srrBA</i>	MW1445 MW1446	SSP1260 SSP1261	Anaerobic respiration metabolism, growth at low temperature
<i>phoRP</i>	MW1636 MW1637	SSP1073 SSP1074	Phosphate uptake and homeostasis
<i>airRS</i>	MW1789 MW1790	SSP0946 SSP0947	Oxidative stress response
<i>vraRS</i>	MW1824 MW1825	SSP0908 SSP0909	Cell wall-affecting antibiotic resistance, cell wall biosynthesis
<i>agrCA</i>	MW1962 MW1963	SSP0839 SSP0840	Quorum sensing control of adhesion and virulence factors
<i>kdpDE</i>	MW2002 MW2003		Potassium homeostasis regulation
<i>hssRS</i>	MW2282 MW2283	SSP0540 SSP0541	Heme metabolism regulation
<i>nreCB</i>	MW2313 MW2314		Response to low oxygen, nitrate reduction
<i>braSR</i>	MW2544 MW2545		Antimicrobial peptide resistance

persistent kidney infections [33]. Another specific feature of uropathogenicity found in *S. saprophyticus* is the presence of a D-serine deaminase responsible for degrading high concentrations of D-serine present in the urine [34]. Regulation of the expression of all these colonization factors in *S. saprophyticus* is not integrated under a single TCS. Instead, *S. saprophyticus* has more specific regulatory genes that may act individually for the modulation of such adhesion factors and metabolic enzymes for a prompt and individual response in metabolite-rich urine.

NreCB

Under conditions of low oxygen tension, *S. aureus* uses nitrate and nitrite as its final oxygen acceptors. NreCB is an oxygen sensing system that activates the expression of the cluster of genes needed for nitrate reduction (*narGHJI*) and nitrite reduction (*nirRBD*) [35,36] (Figure 1). Activation of NreCB is controlled by the nitrate-sensing NreA protein, which inhibits NreB

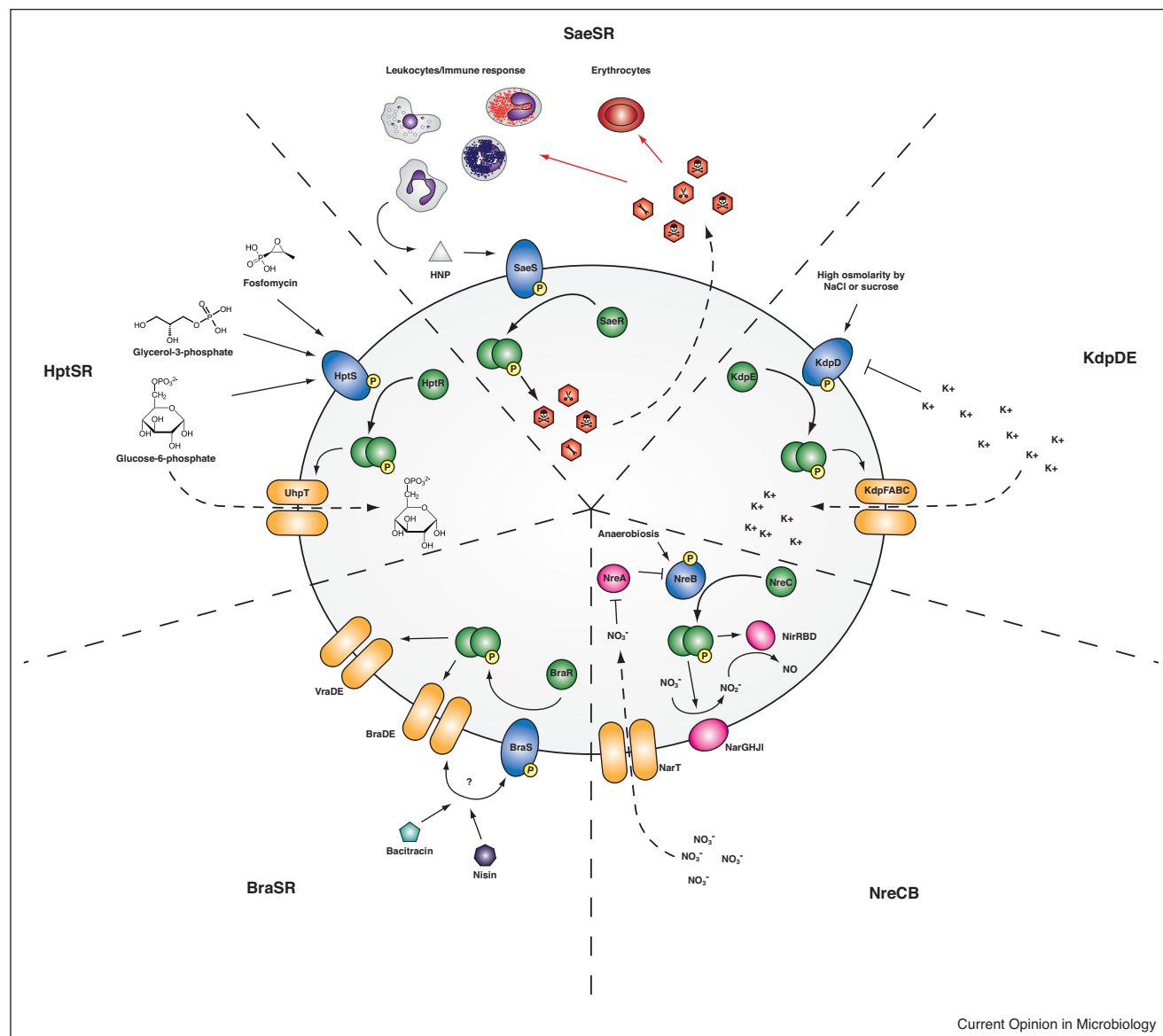
autophosphorylation in the absence of nitrate (via binding to NreB) [36]. Inactivation of NreCB abrogates the ability of *S. aureus* to reduce nitrate, forcing the bacterium to upregulate fermentative pathways for survival [5]. The *nreCB* genes are transcribed together with the nitrate reductase genes (*narGHJI* operon) which is located downstream the genes encoding the nitrite reductase. Also located within the vicinity of *nreCB* is the nitrate transporter encoding gene (*narT*). This genome organization strongly suggests that *nreCB* belongs to a gene cluster that has been acquired by horizontal gene transfer. *S. saprophyticus* cannot use nitrate and nitrite as final oxygen acceptors because of the lack of respiratory nitrate reductase NarGHJI and assimilatory nitrite reductase NirRBD as well as the corresponding NreCB TCS on the genome. An exception to this is *S. saprophyticus* subsp. *bovis*, a regular colonizer of bovine nostrils that contains the NreCB TCS and also the nitrate but not the nitrite reductase genes on what appears to be a transposable element [37]. Another consequence of the nitrate and nitrite reductase activities is the generation of ammonia that allows nitrogen to be converted to an organic form. *S. saprophyticus* produces a potent urease that can obtain ammonia from urea (Figure 2). The urease activity of *S. saprophyticus* is significantly higher than other staphylococcal species and it is required for persistent infection in the urinary tract [38]. Besides, the urease activity is responsible for raising the pH of human urine, which allows precipitation of normally soluble polyvalent ions to carbonate apatite. These compounds aggregate around bacteria and very often are the cause of the formation of urinary stones.

BraSR

BraSR (bacitracin resistance associated) is associated with resistance to the antimicrobial peptides, nisin and bacitracin [39–42]. BraSR forms a module with the ABC transporter BraDE. The ABC transporter is involved in sensing the signal and participates by mechanisms that still are not completely understood in the phosphotransfer between BraS to BraR [43]. In this system, the ABC transporter and the TCS have an absolute mutual requirement for each other in both sensing and responding [44•]. BraSR also activates the expression of VraDE, which encodes for the cognate ABC transporter associated with GraRS (Figure 1).

BraSR is absent in several staphylococcal species including *S. saprophyticus*, *Staphylococcus xylosus*, *Staphylococcus arlettae*, *Staphylococcus intermedius*, and *Staphylococcus pseudointermedius* [44•]. The absence of BraSR in *S. saprophyticus* is likely compensated by the presence of its orthologous TCS, GraRS. Indeed, experimental evolution of *S. saprophyticus* in the presence of increasing concentration of nisin, confirmed the role of GraRS in nisin resistance mechanisms [44•]. In *S. aureus*, activation

Figure 1

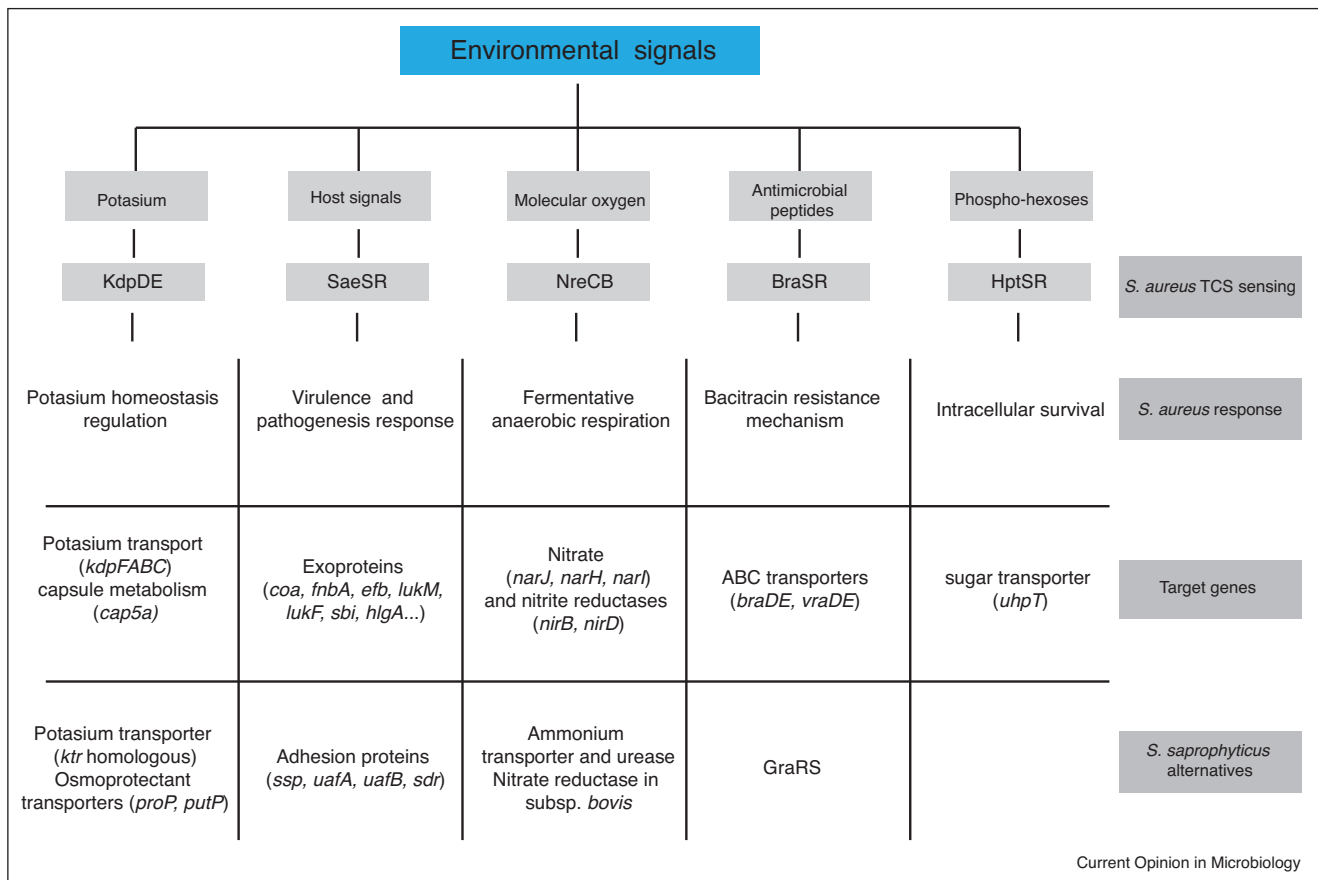


TCSs absent in *S. saprophyticus* and their main functional roles. Five of the 16 TCSs in *S. aureus* are absent in *S. saprophyticus*. The HptSR TCS responds to extracellular phosphates and activates the glucose-6-phosphate transporter UhpT but can also facilitate the uptake of the antibiotic Fosfomycin. SaeSR controls the expression of secreted toxins and immune evasion factors involved in erythrocyte and leukocyte lysis as well as modifying several immune evasion pathways. SaeSR responds to antimicrobial peptides such as the human neutrophil peptide (HNP) secreted by neutrophils. The KdpDE TCS is involved in the control of a highly specific potassium uptake system (KdpFABC) required for growth under highly potassium restricting conditions. The TCS is repressed by potassium ions and cyclic di-AMP and can be activated by high osmotic conditions. Activation of KdpDE also leads to the expression of genes involved in the uptake of compatible solutes and sugars, capsule synthesis as well as amino acid biosynthesis and central metabolism. The NreCB TCS controls the reduction of nitrate to nitrite to nitric oxide. The TCS is inhibited by NreA in the absence of nitrate and induced under anaerobic conditions. The BraSR TCS is involved in the response to antimicrobial peptides such as bacitracin and nisin and involves the BraDE transporter in sensing these peptides. It also activates the expression of the GraRS TCS-associated VraDE transporter.

of GraRS induces the expression *mprF* and the *dltABCD* operon. The *dltABCD* operon contributes to the net positive surface charge by covalently incorporating D-alanine to the cell wall linked teichoic acids whereas MprF adds positively charged lysine residues to

phosphatidyl glycerol within the cell membrane [45]. It is hypothesized that GraRS in *S. saprophyticus* would also induce the expression of *mprF* and the *dltABCD* operon to confer resistance to AMPs (Figure 2). However, this hypothesis has not been experimentally tested.

Figure 2



Summary of the activity of the TCSs absent in *S. saprophyticus*. HptSR, SaeSR, KdpDE, NreCB, and BraSR are the five TCSs present in *S. aureus* but missing in *S. saprophyticus*. *S. aureus* colonises different niches to *S. saprophyticus* and consequently has to adapt to different environmental conditions. The five TCSs specific to *S. aureus* allow it to sense relevant environmental signals and allow it to respond by activating the expression of different target genes. Thus, *S. aureus* uses its larger arsenal of TCSs to adapt its physiology to the new environments. The genome of *S. saprophyticus* encodes genes that may compensate for the loss of genes and pathways regulated by the TCSs absent in this staphylococcal species. HptSR and its function in the uptake of extracellular hexose-6-phosphates is the only TCS-regulated adaptation mechanism for which *S. saprophyticus* does not encode any alternative.

HptSR

HptSR is required to sense extracellular hexose phosphates and to activate the transcription of *uhpT*, a gene located directly downstream *hptSR* that encodes the unique glucose 6-phosphate transporter of *S. aureus* [46] (Figure 1). UhpT is medically relevant because it can mediate the uptake of the antibiotic Fosfomycin [47]. *S. aureus* is currently regarded as a facultative intracellular pathogen [48]. Host cell invasion and intracellular survival is used by *S. aureus* to infect macrophages, spread to secondary points of infection, evade immune recognition, and avoid exposure to last-resort antibiotics [49,50]. To survive and multiply within host cells, *S. aureus*, as many other intracellular pathogens [51], needs to adapt to the available nutrients and other physiological conditions (pH, temperature, oxygen). Since hexose phosphates are abundant carbon sources within the host cell cytosol, the HptSR system is

important for intracellular survival and multiplication of *S. aureus* within host cells (Figure 2). Indeed, *S. aureus* strains deficient in *hptSR* show impaired survival/multiplication within mammalian cells [46]. *S. saprophyticus* is an extracellular pathogen that shows strong adhesion to various epithelial cell lines. Internalization of *S. saprophyticus* has been described in human bladder carcinoma cell lines [46], but the relevance to UTI infection has not been documented. Even if *S. saprophyticus* might get internalized by some epithelial cells during its life cycle, the absence of a system to take up hexose phosphates would impair the replication and multiplication of the bacterium in the cell's cytoplasm. Alternatively, it cannot be excluded that the absence of HptSR might be replaced by a different pathway for the uptake of glucose 6-phosphate or that other nutrients are important for intracellular survival of *S. saprophyticus*. The HptSR system involves four genes that are located together in

the *S. aureus* genome. Thus, complementation of *S. saprophyticus* with the whole system would be the direct approach to determine whether HptSR is sufficient to confer *S. saprophyticus* the capacity to replicate in the cytosol of the host cell.

Conclusion and future directions

S. aureus and *S. saprophyticus* have been classified as high and medium-pathogenic staphylococci, respectively. Medium-pathogenic staphylococci are more specialized in their infective strategies and cause a narrow spectrum of diseases [52]. The reduced number of TCSs is not by itself the reason for a lower pathogenic capacity because many other medium-pathogenic staphylococci (*S. epidermidis*, *S. lugdunensis*, *S. haemolyticus* and *S. pseudointermedius*) have at least the same number of TCSs as *S. aureus*. Instead, the number of TCSs correlates with the presence/absence of those genes encoding for metabolic pathways necessary to adapt bacterial growth to the hostile environment of the host and to harmonize their expression. A question that remains open is whether the common staphylococcal ancestor contained the sixteen TCSs and *S. saprophyticus* suffered genome reduction events during evolution or alternatively, the staphylococcal ancestor contained a lower number of TCSs and *S. saprophyticus*, contrary to other staphylococcal species, did not gain additional TCSs. The mechanisms of TCS acquisition/loss during bacterial evolution have been theoretically predicted but they have not been experimentally addressed. Hence, an effort that considers long-term experiments, in which bacteria gain a selective advantage through the acquisition/loss of a TCS would provide insights into how adaptation to different niches was established over evolutionary time.

Conflict of interest statement

Nothing declared.

CRedit authorship contribution statement

Beatriz Rapun-Araiz: Data curation, Investigation, Methodology, Writing - review & editing. **Andreas F Haag:** Data curation, Investigation, Methodology, Writing - review & editing. **Cristina Solano:** Funding acquisition, Writing - review & editing. **Iñigo Lasa:** Conceptualization, Data curation, Investigation, Methodology, Funding acquisition, Writing - original draft, Writing - review & editing.

Acknowledgements

B.R. is recipient of a PhD grant from Universidad Pública de Navarra. Work in the Laboratory of Microbial Pathogenesis is funded by the Spanish Ministry of Science, Innovation and Universities grant BIO2017-83035-R, Agencia Española de Investigación/Fondo Europeo de Desarrollo Regional, European Union. A.F.H. is supported by the European Research Council ERC under the European Union's Horizon 2020 research and innovation program Grant Agreement ERC-ADG-2014 Proposal n° 670932 Dut-signal from EU awarded to José R. Penadés and was the recipient of a Tenovus Project Grant (S16-12).

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